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LDL Receptor Status of Human Glioblastoma Cells

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1.INTRODUCTION

Porphyrins are known to associate with plasma lipoproteins, particularly low density lipoproteins (LDLs), and are thus likely to be taken up into cells by the high affinity saturable LDL receptor mechanism[1]. We have previously reported that the boronated protoporphyrin, BOPP, is incorporated into the human glioblastoma cell line, SF 767 (derived from a glioblastoma multiforme biopsy specimen obtained from a grade 4 malignant glioma), and requires the presence of lipoproteins for maximum uptake into cells[2]. We concluded from these studies that, like other porphyrins, BOPP forms a complex with LDL before it is taken up into the SF 767 cells via the LDL receptor; however, the presence of saturable LDL receptors on SF 767 cells has not been previously demonstrated. We have therefore investigated whether the LDL receptor was present on exponentially growing SF 767 cells. We report here our initial results indicating relatively high levels of LDL-receptors per cell (over 200,000) from the SF-767 line.

2.MATERIALS AND METHODS

The glioblastoma cell line, SF-767, previously described by Basu et al.[3] was used. Classical LDL binding studies were carried out at 4°C with ^{125}I-LDL in the presence and absence of a 50-fold excess of unlabeled LDL[4]. The latter provides information on the non-specific binding of LDL. To assure that LDL receptors were maximally upregulated, the cells were grown for 24 hr in medium containing lipoprotein deficient serum. The difference between total ^{125}I-LDL binding and non-specific binding provides information on the saturable, specific binding of LDL. LDLs were labeled by the Iodo-Bead method using the instructions provided by the manufacturer (Pierce, Rockford, IL).

3.RESULTS

Figure 1 is a representative example of four binding studies showing binding of LDL to SF 767 cells at 4°C. The figure clearly demonstrates that there is a saturable and specific binding component where saturation occurs at approximately 760 ng ^{125}I-LDL/mg cell protein.

Figure 1. ^{125}I-LDL Binding to SF 767 Cells

The Scatchard plot analysis shown in Figure 2 indicates high affinity binding of LDL to its receptor, where the K_d (binding affinity) is 4.9 nM. From these data we calculate that at 4^{o}C there are approximately 210,000 receptors per cell. To place the latter data in perspective with receptor number on other cell types, we have summarized the K_d and B_{max} (maximal LDL binding) data from several other cell types in Table 1. This information suggests that, compared to fibroblasts and the human hepatoma line, Hep G2, SF 767 cells have a greater B_{max} ; however, the K_d is similar for fibroblasts and SF 767 cells and this affinity is greater than that found in Hep G2 cells. Overall, the data suggest that this glioblastoma cell line has large numbers of LDL receptors that provide a potential mechanism for targeting therapeutic agents to these cells.

Figure 2. Scatchard Plot Analysis of ^{125}I-LDL Binding to SF 767 Cells

Table 1. LDL Receptor Data Reported in the Literature (Binding at 4^{o}C)

4.DISCUSSION AND CONCLUSIONS

It is well known that white blood cells from patients with monocytic or myelomonocytic leukemia have markedly increased LDL degradation compared with cells from control subjects[8]. The rapid receptor-mediated degradation of LDL in leukemia subjects is associated with a hypocholesteremic state in these patients. Interestingly, patients with lymphoblastic leukemia do not have a reduction in plasma cholesterol, nor do the white blood cells exhibit increased binding and uptake of LDL. These data suggest that some tumor cells have increased numbers of high affinity LDL receptors while others do not. High numbers of high affinity LDL receptors could be a mechanism for targeting anti-tumor drugs to specific cell types using LDL as the transport vehicle.

We have recently shown that SF 767 cells take up BOPP and LDL and that both components localize in the lysosome which is the compartment for the degradation of internalized LDL[2]. These findings are suggestive that the LDL receptor is the prime mechanism for delivery of BOPP to this glioblastoma cell. The intracellular localization of BOPP and LDL, however, provided no insights into LDL receptor affinity or receptor number. Without the latter information it is difficult to assess whether the use of LDL receptors for targeting anti-tumor pharmaceuticals (e.g., boronated and/or chemotherapy agents) is a potentially useful therapeutic strategy as suggested by Laster et al.[9] Our results indicate that the SF 767 cell line has relatively high numbers of high affinity LDL receptors. If elevated LDL receptors are a common feature of glioblastoma cells, increasing LDL receptor number by pharmacological means and devising boronated compounds that are recognized by the LDL receptor may be an efficient mechanism for targeting glioblastoma cells for BNCT. A comprehensive survey of LDL receptors on other glioblastoma cell lines is currently underway.

5.ACKNOWLEDGEMENTS
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